

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-65. (Cancelled).

¹²
~~68.~~ (Previously presented) The method of claim ¹~~139~~, wherein said GPCR is a taste receptor.

¹³
~~67.~~ (Previously presented) The method of claim ¹~~139~~, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenicol acetyl transferase, β -galactosidase, β -lactamase and secreted alkaline phosphatase.

¹⁴
~~68.~~ (Previously presented) The method of claim ¹~~139~~, further comprising contacting said cell with a compound that increases calcium levels inside said cell.

¹⁵
~~69.~~ (Original) The method of claim ¹⁴~~68~~, wherein said compound is selected from the group consisting of ionomycin and thapsigargin.

¹⁶
~~70.~~ (Original) The method of claim ¹⁴~~68~~, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

71-73. (Cancelled).

¹⁷
~~74.~~ (Previously presented) The method of claim ²~~140~~, wherein said signal transduction detection system comprises an intracellular calcium indicator.

75-77. (Cancelled).

~~18~~ 78. (Previously presented) The method of claim ~~141~~³, wherein said signal transduction detection system comprises an intracellular calcium indicator.

79. (Cancelled).

~~19~~ 80. (Previously presented) The method of claim ~~141~~³, wherein said detecting comprises fluorescence detection.

81-83. (Cancelled).

~~20~~ 84. (Previously presented) The method of claim ~~142~~⁴, wherein said detecting comprises fluorescence detection.

~~21~~ 85. (Previously presented) The method of claim ~~142~~⁴, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenicol acetyl transferase, β -galactosidase, β -lactamase and secreted alkaline phosphatase.

~~22~~ 86. (Previously presented) The method of claim ~~142~~⁴, further comprising contacting said cell with a compound that increases calcium levels inside said cell.

~~23~~ 87. (Original) The method of claim ~~86~~²², wherein said compound is selected from the group consisting of ionomycin and thapsigargin.

~~24~~ 88. (Previously presented) The method of claim ~~86~~²², further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

89-92. (Cancelled).

²⁵
~~93~~. (Previously presented) The method of claim ~~143~~⁵, wherein said signal transduction detection system comprises an intracellular calcium indicator.

94-96. (Cancelled).

²⁶
~~97~~. (Previously presented) The method of claim ~~144~~⁶, wherein said detecting comprises fluorescence detection.

²⁷
~~98~~. (Previously presented) The method of claim ~~144~~⁶, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenicol acetyl transferase, β -galactosidase, β -lactamase and secreted alkaline phosphatase.

²⁸
~~99~~. (Previously presented) The method of claim ~~144~~⁶, further comprising contacting said cell with a compound that increases calcium levels inside said cell.

²⁹
~~100~~. (Original) The method of claim ~~99~~²⁸, wherein said compound is selected from the group consisting of ionomycin and thapsigargin.

³⁰
~~101~~. (Previously presented) The method of claim ~~99~~²⁸, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

102-104. (Cancelled).

³¹
~~105~~. (Previously presented) The method of claim ~~145~~⁷, wherein said detecting comprises fluorescence detection.

³²
106. (Previously presented) The method of claim ~~146~~⁷, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenicol acetyl transferase, β -galactosidase, β -lactamase and secreted alkaline phosphatase.

³³
107. (Previously presented) The method of claim ~~148~~⁷, further comprising contacting said cells with a compound that increases calcium levels inside said cells.

³⁴
108. (Previously presented) The method of claim ~~107~~³³, wherein said compound is selected from the group consisting of ionomycin and thapsigargin.

³⁵
109. (Previously presented) The method of claim ~~107~~³³, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

³⁶
110. (Previously presented) The method of claim ~~67~~¹³, further comprising contacting said cell with a reporter gene substrate.

³⁷
111. (Previously presented) The method of claim ~~110~~³⁶, wherein said reporter gene is β -lactamase.

³⁸
112. (Previously presented) The method of claim ~~88~~²¹, further comprising contacting said cell with a reporter gene substrate.

³⁹
113. (Previously presented) The method of claim ~~112~~³⁸, wherein said reporter gene is β -lactamase.

⁴⁰
114. (Previously presented) The method of claim ~~98~~²⁷, further comprising contacting said cell with a reporter gene substrate.

⁴¹
~~113.~~ (Previously presented) The method of claim ~~114~~⁴⁰, wherein said reporter gene is β -lactamase.

⁴²
~~116.~~ (Previously presented) The method of claim ~~106~~³², further comprising contacting said cell with a reporter gene substrate.

⁴³
~~117.~~ (Currently amended) The method of claim ~~106~~⁴² ~~116~~, wherein said reporter gene is β -lactamase.

⁴⁴
~~118.~~ (Previously presented) The method of claim ~~111~~³⁷, wherein said reporter gene substrate is CCF2.

⁴⁵
~~119.~~ (Previously presented) The method of claim ~~113~~³⁹, wherein said reporter gene substrate is CCF2.

⁴⁶
~~120.~~ (Previously presented) The method of claim ~~115~~⁴¹, wherein said reporter gene substrate is CCF2.

⁴⁷
~~121.~~ (Previously presented) The method of claim ~~117~~⁴³, wherein said reporter gene substrate is CCF2.

⁴⁸
~~122.~~ (Previously presented) The method of claim ~~141~~³, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

⁴⁹
~~123~~. (Previously presented) The method of claim ~~142~~⁴, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

⁵⁰
~~124~~. (Previously presented) The method of claim ~~143~~⁵, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

⁵¹
~~125~~. (Previously presented) The method of claim ~~144~~⁶, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

⁵²
~~126~~. (Previously presented) The method of claim ~~74~~¹⁷, wherein said intracellular calcium indicator is Fura II.

⁵³
~~127~~. (Previously presented) The method of claim ~~78~~¹⁸, wherein said intracellular calcium indicator is Fura II.

⁵⁴
~~128~~. (Previously presented) The method of claim ~~93~~²⁵, wherein said intracellular calcium indicator is Fura II.

⁵⁵
~~129~~. (Previously presented) The method of claim ~~147~~⁸, wherein said calcium-responsive promoter is NFAT.

⁵⁶
~~130~~. (Previously presented) The method of claim ~~148~~⁹, wherein said calcium-responsive promoter is NFAT.

⁵⁷
~~131.~~ (Previously presented) The method of claim ¹⁰~~149~~, wherein said calcium-responsive promoter is NFAT.

⁵⁸
~~132.~~ (Previously presented) The method of claim ¹¹~~150~~, wherein said calcium-responsive promoter is NFAT.

133. - 138. (Cancelled).

¹
~~139.~~ (Previously presented) A method of identifying a G-protein coupled receptor (GPCR) for a given ligand, the method comprising:

- (i) providing a COS-7 cell, said COS-7 cell comprising,
 - a) a first heterologous inducible CMV promoter operably linked to a heptamerized tet operator and to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,
 - wherein said CMV promoter provides for low level expression of said Gα15 protein prior to induction,
 - wherein induction of said CMV promoter provides for at least a three fold increase in expression of said Gα15 protein, and
 - wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,
 - b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein,
 - c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR, wherein said GPCR is not naturally expressed in said cell, and

d) a constitutive promoter operably linked to a polynucleotide encoding a tetracycline-dependent transactivator;

(ii) contacting said COS-7 cell with said ligand; and

(iii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said ligand with reporter gene expression after addition of said ligand and comparing said change, if any, in reporter gene expression to a change, if any, of reporter gene expression in control COS-7 cells comprising only (a), (b), and (d) prior to and after addition of said ligand, wherein a change in reporter gene expression relative to control COS-7 cells indicates that said GPCR is a receptor for said ligand.

148. ² (Previously presented) A method for identifying a G-protein coupled receptor (GPCR) for a given ligand, the method comprising:

- (i) providing a COS-7 cell, said COS-7 cell comprising,
 - a) a first heterologous inducible CMV promoter operably linked to a heptamerized tet operator and to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,
 - wherein said CMV promoter provides for low level expression of said Gα15 protein prior to induction,
 - wherein induction of said CMV promoter provides for at least a three fold increase in expression of said Gα15 protein, and
 - wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,
 - b) a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR, wherein said GPCR is normally

coupled to either $G\alpha_i$, $G\alpha_s$ or $G\alpha_{12}$ in the absence of said $G\alpha_{15}$ protein,
and wherein said GPCR is not naturally expressed in said cell, and
c) a constitutive promoter operably linked to a polynucleotide
encoding a tetracycline-dependent transactivator;

(ii) contacting said COS-7 cell with said ligand; and

(iii) detecting a change in a signal with a signal transduction detection system
by comparing said signal prior to addition of said ligand with said signal after addition of
said ligand, wherein said signal transduction detection system comprises a dye, and
comparing said change, if any, in signal, with a change in signal, if any, in control COS-7
cells comprising only (a) and (c) prior to and after addition of said ligand, wherein a
change in signal relative to control COS-7 cells indicates that said GPCR is a receptor for
said ligand.

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14x

(Previously presented) A method of identifying a ligand for a given G-protein coupled
receptor (GPCR), the method comprising:

(i) contacting a COS-7 cell with a test chemical, said COS-7 cell comprising,
a) a first heterologous inducible CMV promoter operably linked to a
heptamerized tet operator and to a first polynucleotide encoding a
functional $G\alpha_{15}$ protein having at least 95% sequence homology to SEQ.
ID. NO. 2,

wherein said CMV promoter provides for low level expression of
said $G\alpha_{15}$ protein prior to induction,

wherein induction of said CMV promoter provides for at least a
three fold increase in expression of said $G\alpha_{15}$ protein, and

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

- b) a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR, wherein said GPCR is normally coupled to either Gα_i, Gα_s or Gα₁₂ in the absence of said Gα15 protein, and wherein said GPCR is not naturally expressed in said cell, and
- c) a constitutive promoter operably linked to a polynucleotide encoding a tetracycline-dependent transactivator;

(ii) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said test chemical with said signal after addition of said test chemical, wherein said signal transduction detection system comprises a dye, and comparing said change, if any, in signal, with a change in signal, if any, in control COS-7 cells comprising only (a) and (c) prior to and after addition of said test chemical, wherein a change in signal relative to control COS-7 cells indicates that said test chemical is a ligand for said GPCR.

4
142. (Previously presented) A method of identifying a ligand for a given G-protein coupled receptor (GPCR), the method comprising:

- (i) contacting a COS-7 cell with a test chemical, said COS-7 cell comprising,
 - a) a first heterologous inducible CMV promoter operably linked to a heptamerized tet operator and to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

wherein said CMV promoter provides for low level expression of said Gα15 protein prior to induction,

wherein induction of said CMV promoter provides for at least a three fold increase in expression of said Gα15 protein, and

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

- b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein,
- c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR, wherein said GPCR is not naturally expressed in said cell, and
- d) a constitutive promoter operably linked to a polynucleotide encoding a tetracycline-dependent transactivator; and

(ii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said test chemical with reporter gene expression after addition of said test chemical, and comparing said change, if any, in reporter gene expression to a change, if any, of reporter gene expression in control COS-7 cells comprising only (a), (b), and (d) prior to and after addition of said test chemical, wherein a change in reporter gene expression relative to control COS-7 identifies said test chemical as a ligand for said GPCR.

5
143. (Currently amended) A method for identifying a modulator of signal transduction mediated by G-protein coupled receptor (GPCR) activation in a cell, the method comprising:

- (i) contacting a COS-7 cell with a ligand that in the absence of a test chemical activates signal transduction in said COS-7 cell, said COS-7 cell comprising:
 - a) a first heterologous inducible CMV promoter operably linked to a heptamerized tet operator and to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

wherein said CMV promoter provides for low level expression of said Gα15 protein prior to induction,
wherein induction of said CMV promoter provides for at least a three fold increase in expression of said Gα15 protein, and
wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

- b) a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR, wherein said GPCR is normally coupled to either Gα_i, Gα_s, or Gα₁₂ in the absence of said Gα15 protein, and wherein said GPCR is not naturally expressed in said cell, and
- c) a constitutive promoter operably linked to a polynucleotide encoding a tetracycline-dependent transactivator;

(ii) contacting said cell with said test chemical, and

(iii) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said test chemical with said signal after addition of said test chemical and comparing said change, if any, in signal to a change, if any, of signal in control COS-7 cells comprising only (a) and ~~(b)~~ (c) prior to and after addition of said test chemical, wherein a change in signal relative to control COS-7 cells indicates that said test chemical is a modulator of said ligand-induced GPCR signal transduction.

144. (Currently amended) A method for identifying a modulator of signal transduction in a cell, the method comprising:

- (i) contacting a COS-7 cell with a ligand that in the absence of a test chemical activates signal transduction via a GPCR in said COS-7 cell, said COS-7 cell comprising,

a) a first heterologous inducible CMV promoter operably linked to a heptamerized tet operator and to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

wherein said CMV promoter provides for low level expression of said Gα15 protein prior to induction,

wherein induction of said CMV promoter provides for at least a three fold increase in expression of said Gα15 protein, and

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein,

c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR, wherein said GPCR is not naturally expressed in said cell, and

d) a constitutive promoter operably linked to a polynucleotide encoding a tetracycline-dependent transactivator;

(ii) contacting said COS-7 cell with said test chemical; and

(iii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said test chemical with reporter gene expression after addition of said test chemical and comparing said change, if any, in reporter gene expression to a change, if any, of reporter gene expression in control COS-7 cells comprising only (a), (b), and ~~(c)~~ (d) prior to and after addition of said test chemical,

wherein a change in reporter gene expression relative to control COS-7 cells indicates that said test chemical is a modulator of said ligand-induced GPCR signal transduction.

⁷
145. (Currently amended) A method of profiling a test chemical with respect to whether it is a ligand or not for a given GPCR, comprising the steps of:

(i) contacting a panel of COS-7 cells with said test chemical, said panel of COS-7 cells comprising a plurality of COS-7 cell clones, wherein each COS-7 cell clone differs from the other COS-7 cell clones with respect to a GPCR that is expressed therein, each COS-7 cell clone comprising:

a) a first heterologous inducible CMV promoter operably linked to a heptamerized tet operator and to a first polynucleotide encoding a functional G α 15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

wherein said CMV promoter provides for low level expression of said G α 15 protein prior to induction,
wherein induction of said CMV promoter provides for at least a three fold increase in expression of said G α 15 protein, and
wherein induced expression of said G α 15 protein is sufficient to permit promiscuous coupling to said GPCR,

b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, wherein said second heterologous promoter is directly or indirectly modulated by the activity of said G α 15 protein,

c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR, wherein said GPCR is not naturally expressed in said cell, and

d) a constitutive promoter operably linked to a polynucleotide encoding a tetracycline-dependent transactivator;

(ii) detecting reporter gene expression from each of said COS-7 cell clones in said panel; and

(iii) comparing one or more changes in reporter gene expression among said COS-7 cell clones in said panel before and after addition of said test chemical and comparing said one or more changes, if any, in reporter gene expression to one or more changes, if any, of reporter gene expression in a corresponding panel of control COS-7 cells comprising only (a), (b), and (c) (d) prior to and after addition of said test chemical, wherein a change in reporter gene expression relative to a corresponding control COS-7 cell clone in said corresponding panel of control COS-7 indicates that said test chemical is a ligand for the GPCR of the COS-7 cell clone.

146. (Cancelled).

⁸
~~147~~. (Previously presented) The method of claim ¹~~139~~, wherein said second heterologous promoter is a calcium-responsive promoter.

⁹
~~148~~. (Previously presented) The method of claim ⁴~~142~~, wherein said second heterologous promoter is a calcium-responsive promoter.

¹⁰
~~149~~. (Previously presented) The method of claim ⁶~~144~~, wherein said second heterologous promoter is a calcium-responsive promoter.

¹¹
~~150~~. (Previously presented) The method of claim ⁷~~145~~, wherein said second heterologous promoter is a calcium-responsive promoter.